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Microbial incorporation of nitrogen in stream detritus

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Abstract

We adapted the chloroform fumigation method to determine microbial nitrogen (N) and microbial incorporation of ^{15}N on three common substrates [leaves, wood and fine benthic organic matter (FBOM)] in three forest streams. We compared microbial N and ^{15}N content of samples collected during a 6-week ^{15}N–NH_4 tracer addition in each stream. The ^{15}N was added during late autumn to Upper Ball Creek, a second-order stream at the Coweeta Hydrologic Lab, North Carolina, U.S.A.; during spring to Walker Branch, a first-order stream on DOE's Oak Ridge National Environmental Research Park, Tennessee; and during summer to Bear Brook, a first-order stream in the Hubbard Brook Experimental Forest, New Hampshire. FBOM was the largest component of organic matter and N standing stock in all streams. Microbial N represented the highest proportion of total N in leaves and least in FBOM in Walker Branch and Bear Brook. In Upper Ball Creek, the proportion of microbial N was higher in FBOM than in used biofilm or on leaves. Standing stock of microbial N on leaves and in FBOM ranged from 37 mg N m^{-2} in Bear Brook to 301 mg N m^{-2} in Walker Branch. Percent of detrital N in living microbial cells was directly related to total microbial biomass (fungal and bacterial biomass) determined from microscopic counts. $\partial^{15}N$ values for microbes were generally higher than for bulk detritus, which would result in higher $\partial^{15}N$ values for animals preferentially consuming or assimilating microbial cells. The proportion of ^{15}N taken up by detritus during the ^{15}N experiments that remained in microbial cells by the end of the experiments was highest for wood biofilm in Upper Ball Creek (69%), leaves in Walker Branch (65%) and FBOM in Upper Ball Creek (31%). Lower retention proportions (<1–25%) were observed for other substrates. Our results suggest that microbial cells associated with leaves and wood biofilm were most active in ^{15}N–NH_4 immobilization, whereas microbial cells associated with FBOM immobilized little ^{15}N from stream water.

Introduction

Understanding the cycling and retention of nitrogen (N) in stream ecosystems is important since nitrogen is essential for sustaining biological production. Human activities have altered the nitrogen (N) cycle on local, regional and global scales through increased use of fertilizers, poor grazing practices and increased atmospheric N deposition (Vitousek et al., 1997). so understanding uptake and cycling of N in streams has become increasingly more critical. The ability to predict the consequences of increased N inputs to aquatic ecosystems will depend, in part, on our understanding of basic microbial transformations of N, as microbes are among the first organisms to utilize dissolved N, thereby making that N available to higher trophic levels. Also, microbial N uptake will influence the quantity and timing of N delivery to downstream systems.

Until recently, our ability to assess N uptake and

transfer from microbes to higher food web compartments has been limited because of the difficulty in quantifying microbial N, particularly in shaded streams, where microbes colonizing allochthonous organic matter are a significant food resource (Hall & Meyer, 1998). Because microbial N is a highly available form of N for detritivores, isolating microbial N from bulk detrital N is essential for understanding trophic transfers of N in streams (Hall et al., 1998).

Use of ^{15}N tracer additions have enabled stream ecologists to study nitrogen cycling and identify food web linkages in streams without increasing the overall concentration of dissolved nitrogen (Fry et al., 1995; Peterson et al., 1997; Hall et al., 1998; Wollheim et al., 1999). Enough ^{15}N labeled nitrogen is added to the stream to increase ^{15}N:^{14}N ratios, while not increasing overall N concentrations (Peterson & Fry, 1987). Here we report on incorporation of ^{15}N into detritus-associated microbes as part of a larger ^{15}N tracer study [Lotic Intersite Nitrogen Experiment (LINX), Mulholland et al., 2000a].

We modified the chloroform fumigation method commonly used to measure microbial N in soils (Brookes et al., 1985a,b) to isolate microbial N and microbial ^{15}N from stream detritus. Chloroform fumigation kills and lyses microbial cells, and when followed by extraction with K_2SO_4 (Brookes et al., 1985a,b), may be an efficient way to isolate microbial N from other pools of N on stream substrates. We measured ^{15}N values of microbes on FBOM, wood and leaves in three temperate forest streams to separate actively cycling pools of microbial N from bulk N in these substrates.

Study sites

Upper Ball Creek (UBC) is a 2nd-order stream located at the Coweeta Hydrologic Laboratory (a Long-term Ecological Research site) in the Southern Appalachian mountains of western North Carolina, U.S.A. The ^{15}N–NH_4 tracer addition at Upper Ball Creek (UBC) began just after leaf fall on 1 November and ended 16 December 1996 (Tank et al., 2000). Upper Ball Creek has relatively low N and P concentrations, is highly heterotrophic and has the highest discharge and lowest temperature of the three streams studied (Table 1). The dominant substrate in the experimental reach is cobble-riffle with larger pool areas found behind debris dams. The riparian vegetation is predominately deciduous: oak (*Quercus* spp.), hickory (*Carya* spp.) and yellow poplar (*Liriodendron tulipifera*) in the upper canopy; with predominantly rhododendron (*Rhododendron maximum*) and some mountain laurel (*Kalmia latifolia*) in the shrub layer, forming a closed canopy over the stream throughout the year.

Table 1. Physical, chemical and biological characteristics of Upper Ball Creek, Walker Branch and Bear Brook. All values are from the beginning of the ^{15}N tracer additions, except temperature and chemical concentrations, which represent means over the 6 week period of each release

	Upper Ball Creek	Walker Branch	Bear Brook
Physical			
Stream order	2	1	2
Discharge (l/s)	51.4	9.8	3.5
Mean width (m)	2.7	3.1	1.0
Mean depth (cm)	18	4.6	9
Average slope (%)	17	3.5	14
Temperature (°C)	7.2	12.4	14.5
Chemical			
NH_4 (μg N/L)	2	4	4
NO_3 (μg N/L)	1	19	57
SRP (μg P/L)	2	2	8
Metabolism			
GPP ($gO_2\ m^{-2}\ d^{-1}$)	0.06	1.2	0.2
R ($gO_2\ m^{-2}\ d^{-1}$)	32.3	4.0	9.1
P:R ratio	0.002	0.3	0.02

Walker Branch (WB) is a 1st-order stream located on the U.S. Department of Energy's, Oak Ridge National Environmental Research Park, Oak Ridge, Tennessee in the ridge and valley physiographic province of the Southern Appalachian mountains, U.S.A. The ^{15}N–NH_4 tracer addition was carried out from 1 April to 13 May 1997, beginning before leaf-out and ending after canopy closure. Values for both discharge and temperature are intermediate compared to the other two streams studied (Table 1). Walker Branch has relatively low N and P concentrations, but the highest primary productivity of the three streams (Table 1). The dominant substrate in the experimental reach is cobble-riffle, with some bedrock outcrop areas and pools with fine-grained organic rich sediments. Riparian vegetation is mainly deciduous and dominate trees species include: several species of oak (*Quercus* spp.), yellow poplar (*Liriodendron tulipifera*) and red maple (*Acer rubrum*).

Bear Brook (BB) is a 2nd-order stream at the Hubbard Brook Experimental Forest, New Hampshire (a Long-term Ecological Research site). The ^{15}N–NH_4 tracer addition took place from 17 June to 29 July 1997, a period of summer low flow in this stream. Bear Brook had low concentrations of P, relatively high concentrations of N, low primary production and the warmest temperatures during the experimental release (Table 1). The stream bottom consists primarily of bedrock and large boulders; large debris dams are common. The riparian vegetation is dominated by American beech (*Fagus grandifolia*), yellow birch (*Betula lutea*) and sugar maple (*Acer saccharum*).

Materials and methods

Field sampling

We performed a continuous 6-week addition of 10% ^{15}N–NH_4Cl to each stream in order to achieve an enrichment of ^{15}N of approximately 500%, while maintaining background concentrations of NH_4. The solute was released from a 20 l Nalgene carboy connected to a peristaltic pump (Mulholland et al., 2000a). The ^{15}N–NH_4 release rate varied among the three sites (WB=0.1807 mg ^{15}N h^{-1}; UBC=0.6082 mg ^{15}N h^{-1} and BB=0.1886 mg ^{15}N h^{-1}) and was based on stream discharge and background ammonium concentrations. Locations of longitudinal sampling stations in the stream channels were determined by the ammonium uptake lengths measured at the start of the experiment (Webster & Ehrman, 1996). Sampling of the detritus for microbial N and ^{15}N was conducted on day 38 of each release. Based on an uptake length of 25 m at WB, we collected samples on leaves, wood and FBOM 10 m upstream (−10) and 10, 25 and 50 m downstream from the dripper; with an uptake length of 65 m at UBC, we sampled at −10, 27 and 115 m; and based on an uptake length of 104 m at BB, we sampled at −5, 20 and 60 m. The −5 (BB) and −10 m (WB and UBC) stations were unlabeled by the ^{15}N addition and served as references for measuring background ^{15}N values. In Upper Ball Creek, additional samples for determination of microbial N were collected on days 7 and 21 at all stations, and at 50 m on day 21 for ^{15}N analysis.

Leaves were collected from five randomly-chosen areas within 5 m of each station and placed into ziploc bags. Samples were kept moist with stream H_2O until reaching the lab, since microbial cells are less affected by the chloroform fumigation process once dry (Sparling & West, 1988). Similarly, medium to small pieces (<3 cm in diameter) of decomposing woody debris were collected randomly at each of the stations and placed in ziploc bags with water. In the laboratory, wood biofilm was removed by scraping the wood surface. Slurries of surface FBOM were collected from areas of fine-grained sediment accumulation at each sampling station using a suction device and placed into cups.

Additional samples of each type of detritus were collected to obtain standing stocks and C:N ratios. To obtain standing stocks, we used a stratified randomized sampling design to sample habitat types (riffle/run, bedrock outcrop, pool/backwater) in proportion to their actual occurrence (Mulholland et al., 2000a). Detritus was sampled at each Station by placing an open-ended metal cylinder (0.07 m^{-2}) into the sediment as deeply as possible and removed coarse organic matter which was then separated into wood and leaves. We then agitated the upper sediment and pumped it through a 1 mm mesh net into a 20 l bucket. The material in the net was also separated into wood and leaves. In order to estimate FBOM, a subsample was collected from the bucket onto a pre-combusted glass fiber filter (Whatman GFF). Organic matter was dried at 60 °C for 1 week, weighed, combusted at 550 °C for 4 h, rewetted, dried and reweighed for calculation of dry mass (DM) and ash-free dry mass (AFDM). Average standing stock for each type of detritus was determined for each habitat and then weighted by the proportion of that habitat in each stream reach (Mulholland et al., 200a). N content of detritus was determined using the Dumas combustion method (Carlo Erba CN analyzer model 1500). Total standing stock of N in each of the three detrital compartments was determined from N content (mg N g^{-1} DM), measures of standing stock (g AFDM m^{-2}), and measured ratios of AFDM–DM.

Extraction of microbial nitrogen

Microbial N in stream detritus was extracted using the chloroform fumigation method, originally developed for soils (Brookes et al., 1985a,b). In the laboratory, fresh stream samples were weighed and split into two subsamples, one for fumigation and one for an unfumigated control. Subsample sizes were approximately 40 g for FBOM and 10 g for leaves or wood. The first subsample was placed into a 125 ml flask and then into a desiccator, which contained 40 ml of $CHCl_3$ and 3–5 boiling chips. The desiccator was evacuated using a

vacuum pump, causing the $CHCl_3$ to boil vigorously for 2–3 min, after which the desiccator was sealed and left in the dark for 24 h at room temperature. Replicate subsamples were placed in a dessicator without chloroform to serve as non-fumigated controls. The fumigated and non-fumigated controls were removed from the desiccators, then extracted with 60 ml of 0.5 M K_2SO_4. Samples were shaken vigorously on an oscillating shaker for 30 min, then filtered (Whatman 42) into 60 ml Nalgene bottles and frozen for later analysis of N.

During initial trials in Upper Ball Creek, we performed pre-extractions using 0.05 M K_2SO_4 to determine if any significant labile (exchangeable) inorganic pools of adsorbed nitrogen were present in our samples. Because pre-extracted samples contained an insignificant amount of adsorbed inorganic nitrogen, we omitted this step from subsequent extractions.

Following fumigation, total N ($TN_{microbial}$) was determined by converting all N in the K_2SO_4 extracts to nitrate using the alkaline persulfate oxidation method (Cabrera & Beare, 1993). Nitrate concentrations were determined using automated NO_3 analysis (Alpkem or TRAACS 800 autoanalyzer).

Total nitrogen in microbial cells ($TN_{microbial}$) was determined using the following equation:

$$TN_{microbial} = (TN_{fumigated} - TN_{unfumigated}) * 1.85 \quad (1)$$

where $TN_{fumigated}$ is total nitrogen in $CHCl_3$-fumigated samples and $TN_{unfumigated}$ is TN in non-fumigated controls. A correction factor of 1.85 was used to account for incomplete release of microbial biomass N during the 24 h fumigation period (Brookes et al., 1985b). Microbial N mass was normalized to substrate AFDM using wet mass/AFDM conversions determined for each sample type. Microbial N as a percent of total N was calculated using microbial N g^{-1} AFDM values and total N g^{-1} DM determined from C:N analysis and DM/AFDM conversions.

To prepare samples for ^{15}N analysis, NO_3 in the persulfate-oxidized samples was reduced to NH_3 under basic conditions (with the addition of MgO) using DeVarda's alloy (Brooks et al., 1989; Stark & Hart, 1996). The ammonia was allowed to diffuse into the headspace of a closed container and sorbed on acidified filter packets made by placing acidified (30 μl of 2 M H_2SO_4) glass fiber filters (Whatman GF/D) between teflon membranes. Samples were diffused for 5 days at room temperature after an initial 48 h incubation at 60 °C to ensure complete recovery (Brooks et al., 1989; Stark & Hart, 1996). Filters were analyzed for ^{15}N incorporation by high temperature direct combustion and cryogenic separation using a Finnigan delta S Isotope Ratio Mass Spectrometer at the Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.

Calculations of microbial and detrital ^{15}N

We calculated bulk detrital $\partial^{15}N$ and microbial $\partial^{15}N$ values for FBOM, leaves and wood biofilm. All $\partial^{15}N$ values are calculated as:

$$\partial^{15}N = [(R_{sample}/R_{standard}) - 1] * 1000 \quad (2)$$

where $R_{sample}=^{15}N/^{14}N$ ratio in the sample, and $R_{standard}=^{15}N/^{14}N$ ratio in the atmosphere (0.003663) (Peterson & Fry, 1987). All reported $\partial^{15}N$ values were corrected for background ^{15}N content by subtracting $\partial^{15}N$ values of similar samples collected upstream from the ^{15}N addition site. Hence all reported $\partial^{15}N$ values represent only tracer ^{15}N.

Microbial $\partial^{15}N$ associated with each detrital fraction was calculated using the following equation:

$$\partial^{15}N_{microbes} = [(\partial^{15}N_{fumigated} * TN_{fumigated}) - (\partial^{15}N_{unfumigated} * TN_{unfumigated})]/TN_{microbial} \quad (3)$$

where $\partial^{15}N_{fumigated}$ and $\partial^{15}N_{unfumigated}=\partial^{15}N$ of fumigated and unfumigated samples, respectively, and $TN_{fumigated}$ and $TN_{unfumigated}$=total N in fumigated and unfumigated samples, respectively (mg N g^{-1} AFDM). $TN_{microbial}$ is derived from Equation (1).

To determine the fraction of detrital ^{15}N accumulated by microbes, microbial $\partial^{15}N$ values were compared to $\partial^{15}N$ values for bulk detrital pools according to the following equation:

$$CMN = \left[\frac{(\partial^{15}N_{microbes}/1000) * 0.003663 * TN_{microbial}]}{[(\partial^{15}N_{detritus}/1000) * 0.003663 * TN_{detritus}}\right] \quad (4)$$

where CMN is the fraction of the detrital pool that is cellular microbial N, $\partial^{15}N_{detritus}$ is the $\partial^{15}N$ of detritus (background-corrected), and $TN_{detritus}$ is the total detrital N (mg N g^{-1} AFDM).

Fungal biomass and bacterial cell counts

Approximately 2 weeks prior to ^{15}N additions, we collected 5 samples each of CBOM (leaves and wood) and surface FBOM and determined fungal biomass and bacterial abundance (except for fungal biomass samples from Walker Branch which were resampled one year after the ^{15}N experiment under similar conditions). Leaves, wood and FBOM were collected by hand and 5–15 g wet mass were preserved in 20 ml of 5% buffered formalin (for bacterial counts), and 20 ml of HPLC-grade methanol (for ergosterol extraction). Bacterial abundance was estimated from acridine-orange direct counts following homogenization and sonification. Fungal biomass was estimated by measuring ergosterol content of leaves, wood biofilm and surficial FBOM. Ergosterol was extracted at 80 °C for 2 h then saponified, separated in pentane and quantified on a Waters High-Performance Liquid Chromatograph (Newell et al., 1988). For direct comparison of microbial biomass, bacterial cell densities and ergosterol measurements were converted to units of carbon. Bacterial cell numbers were converted to carbon units assuming an average carbon content of 2×10^{-14} g C $cell^{-1}$. This conversion factor is equivalent to a mean cell biovolume of 0.2 μm^3 $cell^{-1}$ and 100 fg C μm^{-3} (Findlay et al., in review). Ergosterol was converted to fungal carbon assuming 1 mg fungal C/11 μg ergosterol (Gessner & Chauvet 1993).

Results

Standing stock of organic matter and total nitrogen

Standing stocks of both detritus and autotrophs were highest in Walker Branch (Table 2). Autotrophic biomass was lowest in Upper Ball Creek and standing stock of detritus was lowest in Bear Brook. Detritus comprised most of the organic material in all three streams (Table 2); leaves, wood and FBOM accounted for 99% of total standing stock of organic matter in Upper Ball Creek, whereas Walker Branch and Bear Brook contained slightly more autotrophic (epilithon and bryophytes) biomass (10 and 17%, respectively). Filamentous algae were not observed in any significant amount in any of the streams. Because autochthonous organic matter was so rare in Upper Ball Creek, detrital N was a larger proportion of total N standing stocks in Upper Ball Creek (98%) than in Walker Branch (73%) or Bear Brook (53%) (Table 2).

Standing stock of FBOM in Bear Brook was an order of magnitude greater than standing stocks of leaves or wood (Table 2). Similarly, FBOM standing stock in Walker Branch was nearly double standing stocks of wood or leaves, although some of the FBOM in Walker Branch may include sloughed algae (Mulholland et al., 2000a). In contrast, leaves dominated detrital standing stock in Upper Ball Creek. Our estimates of wood did not include large debris dams; hence, we have underestimated the contribution of wood to standing stock biomass in Upper Ball Creek and Bear Brook where large debris dams occur.

Differences in standing stocks of allochthonous organic nitrogen in the three streams were a consequence of differences in the quantity of organic matter present and its N content (Table 2). The N:C ratios of FBOM was similar in all streams and higher than in any of the other detrital compartments. Most of the detrital N in all three streams was found in FBOM (Table 2). The amount of N in small woody debris was similar in all streams, so that the N standing stock of wood is a reflection of the amount of wood present. Despite the fact that N content of leaves in Bear Brook was double that in the other streams, leaf N was a smaller fraction of total N in Bear Brook because so few leaves were present.

Microbial N in detritus

To examine temporal variability in microbial N, we compared microbial N in leaves, wood biofilm and FBOM collected from three stations on days 7, 21 and 38 of the Upper Ball Creek experiment. Microbial N content of leaves, wood biofilm, or FBOM did not differ over time (ANOVA, $p>0.05$, Table 3).

Microbial N (mg N g $AFDM^{-1}$) was highest on leaves in Walker Branch and Bear Brook (Table 4) and accounted for considerably greater fraction of detrital N than the other substrates (Table 5). The microbial N fraction on leaves was lowest on freshly fallen leaves in Upper Ball Creek (Table 5). The fraction of wood biofilm N in microbial cells was intermediate in value and similar among sites. Microbial cells generally accounted for the smallest fraction of N in FBOM, with the exception of Upper Ball Creek. These patterns in microbial N appear to be related to total microbial bio-

Table 2. Standing stock of organic matter (g AFDM m^{-2}) and nitrogen (g N m^{-2}) of sampled stream compartments and N:C ratio (gN/gC) collected at the beginning of each ^{15}N tracer experiment

	Upper Ball Creek			Walker Branch			Bear Brook		
	AFDM	N	N:C	AFDM	N	N:C	AFDM	N	N:C
Detritus									
Leaves	62.5	0.81	0.020	76	1.03	0.021	4.2	0.13	0.047
Wood	2.8	0.02	0.022	112	1.10	0.021	2.7	0.03	0.027
FBOM	43.6	1.03	0.051	197	2.71	0.054	46.5	0.74	0.056
Total detritus	108.9	1.86		385	4.84		53.4	0.09	
Autotrophs									
Epilithon	1.3	0.02	0.226	3.8	0.07	0.044	2.7	0.04	0.136
Bryophytes	0.06	0.003	0.047	40	1.69	0.70	9.9	0.75	0.043
Total autotrophs[a]	0.11			41.2			10.6		

[a]Total autotroph biomass is computed as epilithon chlorophyll mass multiplied by 100 (to convert to AFDM) plus AFDM of bryophytes. No filamentous algae were present.

Table 3. Mean microbial N (gN g^{-1} AFDM) on three detrital substrates (wood biofilm, leaves and fine benthic organic matter) from three sampling stations on each date in Upper Ball Creek (n = 3 per date/substrate type).

	Microbial N mg N g^{-1} AFDM (SE)	F ratio (probability $>F$)
Leaves		
Day 7	0.25 (0.12)	2.65 (0.14)
Day 21	0.51 (0.13)	
Day 38	0.68 (0.15)	
Wood		
Day 7	0.32 (0.03)	2.48 (0.16)
Day 21	0.42 (0.02)	
Day 38	0.59 (0.14)	
FBOM		
Day 7	0.95 (0.24)	1.43 (0.31)
Day 21	0.74 (0.35)	
Day 38	1.56 (0.44)	

Table 4. Microbial N normalized to AFDM (mg N g^{-1} AFDM) for three detrital substrates collected in Upper Ball Creek, Walker Branch and Bear Brook. Wood consists of outer layer of wood (approximately 1–2 mm) only.

Biomass	Microbial N (mg N g $AFDM^{-1}$)		
Compartment	Upper Ball Creek	Walker Branch	Bear Brook
Leaves	0.37 (0.09)	2.19 (0.30)	2.43 (0.79)
Wood biofilm	0.32 (0.02)	1.01 (0.12)	0.80 (0.18)
FBOM	0.84 (0.20)	0.92 (0.012)	0.94 (0.44)

mass (Table 6), because the percent of microbial N in detritus is significantly correlated with total microbial biomass in that detritus (r^2=0.74, p=0.02).

Combining estimates of microbial N content (Table 4) with measures of standing stock of detritus (Table 2) enables us to calculate standing stock of microbial N for leaves and FBOM in each of the streams (Table 7). Most microbial N was associated with leaves in Walker Branch and with FBOM in Upper Ball Creek and Bear Brook. Because microbial N was estimated for wood biofilm only (see 'Methods') microbial N standing stocks for wood could not be calculated.

Microbial uptake of ^{15}N

By the end of each experiment, ∂15N values of microbes associated with leaves, wood biofilm and FBOM were several times higher than $\partial^{15}N$ of the detrital-microbial complex at Upper Ball Creek and Walker Branch (Table 5). Microbial $\partial^{15}N$ in Bear Brook was also somewhat higher than $\partial^{15}N$ of leaves and wood biofilm, but not for FBOM. Although absolute $\partial^{15}N$ values are highest in Bear Brook, this reflects the high $\partial^{15}N$ values in the water; ^{15}N labeling of microbes relative to the bulk detrital pools was actually least in Bear Brook.

Over 60% of the ^{15}N incorporated by detritus was in microbial cells on leaves in Walker Branch and in wood biofilm in Upper Ball Creek (Table 5). Percentages were somewhat lower (20–30%) for ^{15}N in microbial cells on leaves and FBOM in Upper Ball Creek and on wood biofilm in Walker Branch. ^{15}N in

Table 5. Comparison of $\partial^{15}N$ values on day 38 for three types of bulk detritus (leaves, wood biofilm and FBOM) and for microbial cells associated with that detritus. Del values are background-corrected and are from 27 m in Upper Ball Creek, 25 m in Walker Branch, and 60 m in Bear Brook. Del values from these sites are combined with data from one (UBC and BB) or two (WB) other sites to calculate the percent of detrital N that is in microbial cells compare with the % of detrital ^{15}N in microbial cells. Wood biofilm consists of the outer layer of wood (approximately 1–2 mm) only

	Upper Ball Creek	Walker Branch	Bear Brook
Leaves			
Bulk detrital (leaves) $\partial^{15}N$	18	21	256
Microbe $\partial^{15}N$	73	64	300
Microbial N as % of detrital N	5	22	10
Microbial ^{15}N as % of detrital ^{15}N	20	65	10
Wood biofilm			
Bulk detrital (wood) $\partial^{15}N$	9	13	132
Microbe $\partial^{15}N$	41	31	210
Microbial N as % of detrital N	8	8	8
Microbial ^{15}N as % of detrital ^{15}N	69	25	15
FBOM			
Bulk detrital (FBOM) ^{15}N	6	8	350
Microbe $\partial^{15}N$	25	23	34
Microbial N as % of detrital N	7	3	3
Microbial ^{15}N as % of detrital ^{15}N	31	3	1

Table 6. Fungal and baterial biomass (mg C g^{-1} AFDM) on three detrital substrates in three streams. For direct comparison of microbial biomass, bacterial cell densities and ergosterol measurements were converted to units of carbon (see text for conversion factors)

Stream	Leaves		Wood biofilm		FBOM	
	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
Upper Ball Creek	2.5	0.17	0.6	0.38	0.06	1.2
Walker Branch	25.1	0.11	20.8	0.09	2.1	0.77
Bear Brook	3.8	0.47	0.6	0.39	0.5	0.82

microbial cells made up the smallest fraction of detrital ^{15}N (<15%) on all substrates in Bear Brook and on FBOM in Walker Branch.

Comparing the percent microbial N in detritus with percent microbial ^{15}N in detritus (Table 5) provides insight into the recent activity of microbes in ^{15}N uptake. At both Upper Ball Creek and Walker Branch, microbes associated with leaves and wood biofilm were responsible for much more of the ^{15}N uptake than would be indicated by their contribution to N content of detritus. The high percent of microbial ^{15}N in detritus and high ratios of percent microbial ^{15}N to percent of microbial N suggests a microbial community actively cycling nitrogen.

Discussion

Chloroform fumigation as a tool for stream ecologists

The chloroform fumigation technique, which kills and lyses microbial cells appears to be a useful method for determining the proportion of microbial N relative to total N found in allochthonous organic matter. The microbial ^{15}N technique may be useful in comparing the relative activity of these microbes. Although the chloroform-fumigation method has been reported to extract fungal N less effectively than N associated with bacteria and yeast (Jenkinson, 1976), we found a good correlation of microbial N with total microbial biomass (bacteria and fungi).

Table 7. Total microbial N per m^2 (mg N m^{-2}) for two of the primary detritus compartments (leaves and FBOM) found in the three forested streams. Because microbial N was estimated for wood biofilms only (see 'Methods') microbial N standing stocks for wood could not be calculated

Detritus	Upper Ball Creek mg N m^{-2}	Walker Branch mg N m^{-2}	Bear Brook mg N m^{-2}
Leaves	43	222	13
FBOM	76	79	24
Total	119	301	37

There were striking differences in total amount of microbial N expressed per m^2 of streambed for these three streams (Table 7). This may be a consequence of the seasons in which the samples were taken. Microbial N was highest in Walker Branch in spring; likely a result of highly conditioned leaf material that had entered the stream during autumn leaf-fall. Microbial N was intermediate in Upper Ball Creek, which was sampled during autumn just after peak leaf-fall as microbes were beginning to colonize recently fallen leaves. UBC also contained relatively high standing stocks of FBOM. Bear Brook, with the lowest microbial N standing stocks, was sampled in late summer when there had been little input of fresh detritus since the previous autumn. It follows that FBOM was the major source of microbial N in that stream.

Microbial N was a smaller proportion of detrital N for FBOM (3–7%) than for other types of detritus. These low values suggest that a large portion of the N in FBOM is associated with the organic substrate and not necessarily with microbes colonizing that substrate. FBOM in streams is often considered refractory because it is the product of extensive processing by stream invertebrates (Peters et al., 1989) or erosion of degraded soil organic matter (Sollins et al., 1985). That view is consistent with our results suggesting that microbes associated with FBOM are less active in external N immobilization than are microbes associated with other forms of detritus (Table 6).

The chloroform fumigation technique extracts N from living cells, which include bacteria, fungi and algae. If detritus were heavily colonized by algae, this method would not permit separation of algal N from other microbial forms of N. However, for the detrital substrates sampled in this study, algal biomass was most likely very low.

Microbial uptake of ^{15}N

The use of chloroform fumigation method permits separation of ^{15}N associated with living cells from ^{15}N associated with other components. The detrital ^{15}N not accounted for in living microbial biomass may be the result of microbially derived extracellular ^{15}N, incomplete extraction of microbes (Jenkinson, 1976), or extraction of ^{15}N chemically bound to the detrital matrix.

The three detrital compartments (leaves, wood biofilm and FBOM) in the streams appear to have reached isotopic steady state by Day 38 of the experiments (Tank et al., in press; Mulholland et al., 2000a). For all but one of the detritus samples, $\partial^{15}N$ for microbes was greater than $\partial^{15}N$ for bulk detritus. Hence, organisms that preferentially consume or assimilate microbial cells would be expected to be more highly labeled than bulk detritus. This has been observed for collector–gatherers in two streams at the Coweeta Hydrologic Laboratory (Hall et al., 1998; Tank et al., 2000) and for collector–filterers in Walker Branch (Mulholland et al., 2000b). Variation in $\partial^{15}N$ values for microbes in the three streams is, in part a consequence of differences in labeling of $^{15}NH_4$ in stream water, which was greatest in Bear Brook and least in Upper Ball Creek. It also reflects the fact that microbes are probably deriving different proportions of their N from sources that are less labeled than NH_4 such as nitrate or dissolved organic N, or the detrital substrate itself. For example, at Walker Branch the ratio of microbial $\partial^{15}N$:water $\partial^{15}N$ was substantially lower than 1.0, suggesting considerable microbial uptake of forms of N other than NH_4 (Mulholland et al., 2000a). In all streams, microbial $\partial^{15}N$ values were lowest in FBOM and highest in leaves, indicating that biofilm colonizing leaves may be using NH_4 as a primary N source. This also implies that microbes associated with FBOM may make relatively greater use of other N sources (nitrate and dissolved organic N rather than water column NH_4) compared to leaves.

Decomposing leaves, wood and fine particles are important substrates for microbial colonization in all streams. Microbes in turn, are a significant food resource for detritivores (Hall & Meyer, 1998). The chloroform fumigation method, followed by persulfate oxidation was successful in extracting microbial N from all three substrates in each of our forested streams, and so may be useful in determining the actual food quality of these substrates to detritivores. This method combined with ^{15}N enrichment experi-

ments may bring us one step closer to understanding the role that microbes play in N cycling in streams.

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References

Brookes, P. C., J. F. Kragt, D. S. Powlson & D. S. Jenkinson, 1985a, Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. Soil Biol. Biochem. 17: 831–837.

Brookes, P. C., A. Landman, G. Pruden & D. S. Jenkinson, 1985b, Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17: 837–842.

Brooks, P. D., J. M. Stark, B. B. McInteer & T. Preston, 1989, Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci. Soc. am. J. 53: 1707–1711.

Cabrera, M. L. & M. H. Beare, 1993, Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. am. J. 57: 1007-1012.

Findlay, S. E. G., J. L. Tank, S. Dye, W. B. Bowden, W. Dodds, J. Edmonds, S. K. Hamilton, S. Johnson, W. H. McDowell, P. J. Mulholland & H. M. Valett, In review: Bacterial and fungal biomass in detrital organic matter of streams throughout North America. J. n. am. Benthol. Soc.

Fry, B., D. E. Jones, G. W. Kling, R. B. McKane, K. J. Nadelhoffer & B. J. Peterson, 1995, Adding ^{15}N tracers to ecosystem experiments. In Wada, E. et al. (eds), Stable Isotopes in the Biosphere. Kyoto University Press, Kyoto, Japan: 171–192.

Gessner, M. O. & E. Chauvet, 1993, Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl. Envir. Microbiol. 59: 502–507.

Hall, R. O. & J. L. Meyer, 1998, The trophic significance of bacteria in a detritus-based stream food web. Ecology 79: 1995–2012.

Hall, R. O., B. J. Peterson & J. L. Meyer, 1998, Testing a nitrogen cycling model of a forest stream by using a nitrogen-^{15} tracer addition. Ecosystems 1: 283–298.

Jenkinson, D. S., 1976, The effects of biocidal treatments on metabolism in soil – IV. The decomposition of fumigated organisms in soil. Soil Biol. Biochem. 8: 203–208.

Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wolheim, B. J. Peterson, J. R. Webster & J. L. Meyer, 2000a, Nitrogen cycling in a forest stream determined by a ^{15}N tracer addition. Ecol. Monogr. 70: 471–493.

Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wolheim, B. J. Peterson, J. R. Webster & J. L. Meyer, 2000b, Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a ^{15}N tracer addition. J. n. am. Benthol. Soc. 19: 145–157.

Newell, S. Y., T. L. Arsuffi & R. D. Fallon, 1988, Fundamental procedures for determining ergosterol content of decaying plant material by liquid chromatography. Appl. Envir. Microbiol. 54: 1876–1869.

Peters, G. T., E. F. Benfield & J. R. Webster, 1989, Chemical composition and microbial activity of seston in a southern Appalachian headwater stream. J. n. am. Benthol. Soc. 8: 74–84.

Peterson, B. J., M. Bahr & G. W. Kling, 1997. A tracer investigation of nitrogen cycling in a pristine tundra river. Can. J. Fish. aquat. Sci. 54: 2361–2367.

Peterson, B. J. & B. Fry, 1987, Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18: 293–320.

Sollins, P., C. A. Glassman & C. N. Dahm, 1985, Composition and possible origin of detrital material in streams. Ecology 66: 297–299.

Sparling, G. P. & A. W. West, 1988, Modifications to the fumigation-extraction technique to permit simultaneous extraction and estimation of soil microbial C and N. Commun. Soil Sci. Plant Anal. 19: 327–344.

Stark, J. M. & S. C. Hart, 1996, Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for Nitrogen-15 Analysis. Soil Sci. Soc. am. J. 60: 1846–1855.

Tank, J. L., J. L. Meyer, D. M. Sanzone, P. J. Mulholland, J. R. Webster, B. J. Peterson, 2000. Analysis of nitrogen cycling in a forest stream during autumn using a ^{15}N tracer addition. Limnol. Oceanogr. 45: 1013–1029.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger & G. D. Tilman, 1997, Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7: 737–750.

Webster, J. R. & T. P. Ehrman, 1996, Solute Dynamics. In Hauer, F. R. & G. A. Lamberti (eds), Methods in Stream Ecology. Academic Press, New York: 145–160.

Wollheim, W. M., B. J. Peterson, L. A. Deegan, M. Bahr, J. E. Hobbie, D. Jones, W. B. Bowden, A. E. Hershey, G. W. Kling & M. C. Miller. 1999, A coupled field and modeling approach for the analysis of nitrogen cycling in streams. J. n. am. Benthol. Soc. 18: 199–221.